# fMRI and Granger causality modeling combined to study brain functional connectivity under anesthesia

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**Introduction:** fMRI and its BOLD contrast technique have been successfully used to identify functionally active brain areas in awake humans. Contrary, anesthesia has to be applied in animal fMRI studies interfering with the functional signal. A step further in understanding such modulatory effects on brain function is to analyze the connectivity between activated areas under (different) anesthetics. Granger Causality Mapping (GCM) methods based on vector autoregressive modeling can be used for this purpose (1, 2). In this work we combine GCM and BOLD techniques to better understand the effects of anesthesia on brain function.

**Methods:** *Animal preparation:* Four different groups of Sprague Dawley rats (n>10) were anaesthetized during the experiment with four different anesthetics: Isofluorane (ISO: air mix of 450$O_2$ + 350$N_2$0 ml/min), Isofluorane+$N_2O$ ($N_2O$: air mix of 450$O_2$ + 550$N_2O$ ml/min), Rompun/Ketanest (RK: i.p. injection, 5mg/kg Rompun + 75mg/Kg Ketanest), and Trapanal (TRA: 1ml i.p. injection of 6% Trapanal). Physiological monitoring (respiration rate and $pCO_2$ measurements) was performed during the whole experiment. *Paradigm:* Heat was applied with a peltier element to the left hindpaw. Four different heat stimuli (45,50,55 and 60˚C) were given 3 times with stimulus period of 20 seconds + 200 s rest. Whole brain volumes were acquired during the experiment time (50min). *fMRI:* Scanning was performed on a 4.7T Bruker horizontal magnet (400mT/m) with a quadrature surface coil. After acquiring a scout scan for positioning, fMRI data was acquired with a GE-EPI sequence (2 excitations TR=2×2000 ms, TE effect=24.4 ms, 64×64 matrix, FOV 25 mm×25 mm giving an in-plane resolution of 390×390 µm, 1 mm slice thickness). GLM analysis was performed with BrainVoyager after appropriate preprocessing. Home programmed software (under IDL) was used to calculate average BOLD signal of different functional groups formed by brain structures associated with somatosensory and pain circuits. This groups were: Medial Thalamus (MTh), Lateral Thalamus (LTh), Primary and secondary somatosensory cortex (SI & SII respect.), Association cortex (Assoc), link to limbic system (LinkLS) or limbic system (LS), Hypothalamus (Hth), periaqueductal gray (PAG), Cerebellum (Cereb) and Motor cortex (Motor). *GCM maps*: GCM maps were obtained with a specific Matlab toolbox (Mathworks, Natick, MA) developed by A.K. Seth (3). Data fulfilled in all cases the stationary covariance requisite. Number of lags (measurements prior to time 0) used was 3. This value was a result of Bayesian and Aikaike information criteria calculations. The number of connections (input and output connections per brain region) were obtained from the circular display graphs obtained as outputs to model calculations.

**Results & Discussion:** Figure **1** presents % BOLD activation for four different anesthetic regimes in 11 different functional groups of the rat brain. Figure **2**, present the average GCM connections for the same functional groups and anesthetics as in the BOLD case for the right side brain structures (contralateral to stimulus). Figure **2A,** shows inputs and figure **2B** outputs.

*BOLD discussion:* BOLD responses are larger for the Trapanal anesthetic regime than for the other three anesthetics. The two isofluorane regimes are similar in % BOLD signal and RK shows the least BOLD response. BOLD signal shows no major functional lateralization (data not shown) in agreement with findings from other pain studies (4). *GCM discussion:* ISO, RK and TRA show no output from the medial thalamus (Mth, Fig 2B). This is in accordance with previous studies suggesting for RK that Mth is the area inactive under this drug (5). The other two anesthetics show similar results. ISO also shows lack of connectivity from the cerebellum indicating a blockage of motor output. In general TRA reduces connectivity the most. N2O (laughing gas) produces the opposite effect increasing output and input connectivity. We hypothesize that the effect of this gas is to create a general noisy hyper-connectivity state where pain is not processed properly. *BOLD and GCM discussion:* There is no correlation between BOLD and input or output GCM results. In the case of TRA connectivity is minimal which contrasts with BOLD maximal results. We hypothesize that this lack of correlation is due to limitations on the modeling of the GCM using long TRs fMRI data. GCM calculates its models using data from previous time points of the regions studied (number of lags=number of previous steps analyzed). A TR of 4 s limits our studies to steady state connections lasting more than 4s, but misses probably fast (and strong) connections lasting just a few ms that might induce (strong) BOLD signals. A preliminary analysis shows that TRA has the strongest temporal cross-correlation (data not shown) between the ROIs studied which might explain that it has the worst GCM/BOLD comparability of the four anesthetics.

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**References:** [1] Goebel et al. (2003) MRI 21:1251-61, [2] Roebroeck et al. (2005) NeuroImage 25:230-42, [3] Seth A.K. et al. (2005) Network: Computation in Neural Systems, 16(1):35-55, [4] Shah et al. Psychopharmacology (2005) 180:761-773, [5] Rogers R. Proc. ISMRM 11 (2004)

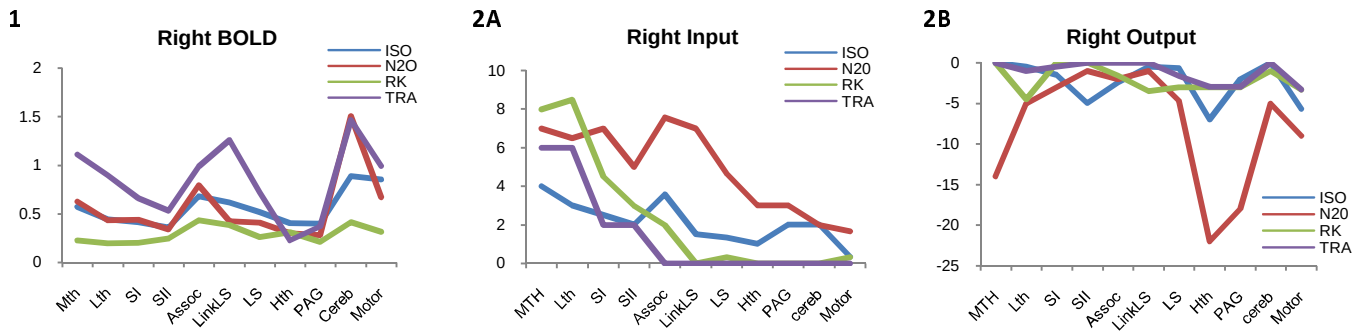


Figure 1:Shows % BOLD response associated with pain for all animals on right side brain structures. Figure 2 shows GCM inputs (2A) and outputs (2B) for all right regions active during pain stimulus.